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Using connectivity to identify climatic drivers of local adaptation

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33   **Abstract**

34   Despite being able to conclusively demonstrate local adaptation, we are still often

35   unable to objectively determine the climatic drivers of local adaptation. Given the

36   rapid rate of global change, understanding the climatic drivers of local adaptation is

37   vital. Not only will this tell us which climate axes matter most to population fitness,

38   but such knowledge is critical to inform management strategies such as translocation

39   and targeted gene flow. Targeted gene flow, for example, requires knowledge of

40   where in the landscape we can find populations with pre-adapted climate-relevant

41   traits; we cannot find these populations if we do not know the dominant climatic

42   drivers of local adaptation. While simple assessments of geographic trait variation are

43   useful, geographic variation — and its associations with environment — may

44   represent plastic, rather than evolved differences. Additionally, the vast number of

45   environment–trait combinations makes it difficult to determine which aspects of the

46   environment populations adapt to. Here we argue that by incorporating a measure of

47   landscape connectivity as a proxy for gene flow, we can differentiate between

48   environment–trait relationships that are under selection versus those that reflect

49   phenotypic plasticity. By doing so, we can rapidly shorten the list of environment–

50   trait combinations that may be of adaptive significance. We demonstrate this method

51   using data on geographic trait variation in a lizard species from Australia's Wet

52   Tropics rainforest.

53

54   **Significance Statement**

55   Despite 150 years of evolutionary research, we still do not have a good method for

56   determining the climatic drivers of local adaptation. Having such a method is critical

57   if we are to understand and mitigate the impact of climate change. Here we exploit the

58   fact that local adaptation is eroded by gene flow to develop a new method for

59   identifying the environmental drivers of local adaptation. Our method will allow

60   workers around the world, for the first time (and often using existing datasets), to

61   determine the climatic drivers of local adaptation in their system. As such, the method

62   has powerful implications not only for pure evolutionary research, but also for

63   management.

64

Introduction

65

66   It is only recently that we have begun to appreciate the speed with which evolution

67   can happen; not only over relatively short timespans (e.g., 1, 2-4), but also at small

68   spatial scales (5). Rapid local adaptation has been recorded in response to a wide suite

69   of environmental drivers, including invasive species, and pollution (6). We expect

70   climate to also be a major driver of local adaptation (e.g., 7, 8), and understanding the

71   way in which species respond to climate is of increasing importance because

72   anthropogenic climate change is proceeding at such a rate that there are concerns that

73   many species will be unable to evolve rapidly enough to avoid extinction (9, 10).

74

75   Evolution typically optimizes phenotypes, but the optimum will vary through both

76   time and space (11, 12), in turn leading to populations ('demes') that have, on average,

77   higher fitness in their home environment than an immigrant would: local adaptation.

78   While adaptive optima for traits almost always vary geographically, it does not follow

79   that all geographic trait variation is due to local adaptation. Geographic trait variation

80   can arise due to other factors, such as phenotypic plasticity (including developmental

81   plasticity and maternal effects) and environmental factors (such as geographic

82   variation in fitness-reducing parasites). These factors, and particularly phenotypic

83   plasticity, can give the appearance of local adaptation, despite having no underlying

84   heritable basis (10, 11), complicating our identification of climate-relevant adaptive

85   variation.

86

87   To circumvent these issues, evolutionary biologists use experimental approaches to

88   demonstrate local adaptation (12, 13). Experiments designed to detect local adaptation

89   typically utilise one of two techniques: 1) reciprocal transplants, which are done *in*

90   *situ*, and are considered the gold standard for demonstrating local adaptation; or 2)

91   common garden experiments, which are usually done in the lab where it is easier to

92   control each environmental variable (12). Both of these techniques can be difficult,

93   for reasons of time, expense, logistics, or ethics. This difficulty increases as the

94   number of separate demes and environmental variables to be tested increases and as

95   the generation time of the organism increases (12). Additionally, although reciprocal

96   transplants will detect signs of local adaptation, they are not necessarily suited to

97   identifying the environmental drivers of that local adaptation (14). This is because *in*

98   *situ* reciprocal transplants necessarily encompass all the environmental variables that

99   differ between the transplant locations. Lab-based common garden approaches may,

100   in principle, be more suited to identifying environmental drivers (because the

101   environment may be under a degree of control), but in practice it often remains

102   impossible to identify the environmental drivers of trait variation seen in the wild.

103   Thus, the best experimental tools we have for studying local adaptation are

104   demanding in terms of time and cost, and are unsuitable for assigning environmental

105   drivers (such as climate variables) to adaptive variation. If we are looking for climate-

106   driven local adaptation, this is a problem: we want to know which climate variable or

107   variables are the main drivers of adaptation, and we urgently need this information for

108   many species.

109

110   By definition, local adaptation has a genetic basis and is consequently weakened by

111   gene flow (11, 15, 16). Demes with high levels of inward gene flow are therefore

112   likely to be less optimally adapted, causing an observable mismatch between optimal

113   and actual phenotypes. Some examples of this are birds dispersing and producing

114   clutch sizes that are not optimised for the habitat quality in which they are now

115   nesting (17), larval salamander colouration not matching streambed colouration due to

116   high levels of gene flow from nearby but predator-free streams (18), and stick insects

117   in smaller habitat patches having non-cryptic colouration when the surrounding

118   patches are larger and environmentally dissimilar (19). These observations of

119   "migrant load" suggest an alternative technique for identifying and assessing local

120   adaptation. First, we look across populations for correlations between the environment

121   (e.g., mean annual temperature) and traits (e.g., morphology, physiology). By

122   themselves, these correlations are not sufficient evidence of local adaptation — they

123   could also be caused by phenotypic plasticity. Second, knowing that local adaptation

124   is hindered by gene flow, we can look at whether gene flow diminishes the

125   environmental effect. In cases where data on gene flow are absent (which is often the

126   case), habitat connectivity can be used as a substitute for gene flow. Environment–

127   trait correlations that are strong, but which are also weakened by connectivity, are

128   indicative of environment–trait correlations that have arisen through local adaptation.

129

130   If we now collect data on a large number of environment–trait correlations, and their

131   interaction with connectivity, we can imagine several possible patterns emerging.

132   These possibilities are depicted in Figure 1. Each panel represents a possible

133   relationship between environment–trait correlations (environmental coefficients on

134   the x-axis) and the interaction between environment and connectivity (y-axis). Panel

135   A shows a set of environment–trait relationships that are strong, but that are not

136   influenced by connectivity (i.e., no environment–connectivity interaction). This

137   pattern is indicative of a system in which environment–trait correlations are

138   predominantly driven by plastic responses of traits to their environment (i.e., traits

139   always match the local environment, regardless of the level of inward gene flow).

140   Plastic responses are still of interest, and many species are likely to show such

141   plasticity as the climate changes (20). Panel B shows a system in which environment–

142   trait relationships are eroded by connectivity: increased connectivity diminishes the

143   correlation between the environment and the trait. In this situation, the interaction

144   between the environmental variable and connectivity is negative when the

145   environmental coefficient is positive (i.e., greater connectivity causes the

146   environmental coefficient to decrease towards zero; bottom-right quadrant), and

147   positive when the environmental coefficient is negative (i.e., greater connectivity

148   causes the environmental coefficient to increase towards zero; top-left quadrant). This

149   is the pattern we would expect if there is a genetic basis to the environment–trait

150   correlation, i.e., local adaptation. Panel C shows the situation where the effect of the

151   environment tends to be enhanced by connectivity. This pattern might arise in

152   organisms that are highly mobile and can actively move to their ideal environment,

153   thus avoiding the selective pressures that would lead to local adaptation.

154

155   Understanding how species respond to specific aspects of their environment is vital if

156   we are to have any hope of halting the current rapid loss of biodiversity. Climate

157   change is undoubtedly one of the biggest threats to global biodiversity (21, 22), and

158   conservation biologists are looking to a variety of techniques to assess and help

159   mitigate the impacts of climate change on vulnerable species (23-25). One technique

160   that is likely to see increasing use is targeted, or assisted gene flow [TGF; for review,

161   see (23, 26)]. This technique involves the spatial redistribution of long-standing

162   adaptations, and acts to increase genetic diversity in recipient populations, thereby

163   bolstering capacity for evolutionary adaptation (10, 23, 25, 26). When applying TGF

164   to help species adapt to climate change, we need to find an existing location that

165   matches the future climate at our recipient site, and then translocate animals from that

166   source location. It is a simple idea, but climate is multidimensional and species will

167   not be adapting equally to each climate axis: is a difference of 0.5°C in mean

168   temperature more important than a difference of 100mm in annual rainfall? The

169   answer depends upon which axes have the strongest influence on fitness.

170

171   Here we explore the idea of using connectivity to infer local adaptation. To do this we

172   develop a case study of a lizard species from northern Australia. We use this system

173   to examine the relationship, across sites, between traits and climatic variables. We

174   assess how habitat connectivity affects these relationships and use the interaction

175   between the environmental variable and connectivity to rank environment–trait

176   combinations. In doing so, we reveal a set of environment–trait relationships

177   dominated by local adaptation.

178

179

180   **Methods**

181   <u>Study species and site selection</u>

182   The Rainforest Sunskink (*Lampropholis coggeri*) is a small (snout–vent length up to

183   45 mm), diurnal scincid lizard restricted to the rainforests of the Wet Tropics region

184   of northeastern Australia (27). The rainforests of this region cover a wide range of

185   environmental conditions, spanning significant elevation (0–1600 m ASL),

186   precipitation (annual mean precipitation of 1432–8934 mm, not including input from

187   cloud stripping), and temperature (annual mean temperature of 16.3–25.8˚C)

188   gradients. This heliothermic skink is active year-round, often seen basking in patches

189 of sunlight on the rainforest floor. Lizards were captured by hand from sites that were

190 selected to maximize the environmental heterogeneity sampled (Fig. 2).

191

192 Morphological measurements were obtained from 532 skinks from 32 sites.

193 Physiological measurements were obtained from a smaller subset of these lizards: 259

194 skinks from 12 sites. Between 8–20 skinks were caught at each site on each collecting

195 trip. Following capture, skinks were transported to James Cook University (JCU) in

196 Townsville for trait measurement. All procedures involving lizards were approved by

197 the JCU animal ethics committee (projects A1976 and A1726).

198
199 Physiological trials

200 Physiological trials commenced within seven days of skinks being collected from the

201 field; skinks being used only for morphology were measured and released back at

202 their point of capture within seven days. The following measures were taken from

203 each skink (n = 259) during laboratory trials: critical thermal minimum (CTmin),

204 critical thermal maximum (CTmax), thermal-performance breadth for sprinting

205 (breadth80), maximum sprint speed (Rmax), temperature at which sprint speed is

206 optimized (Topt), active body temperature as measured in a thermal gradient

207 (Tactive), and desiccation rate (des) (see Table S1 for further details). Details of trait

208 measurement procedures are detailed elsewhere (see 28, 29).

209

210   <u>Morphological measurements</u>

211   The following measurements were taken from each skink (n = 532) using digital

212   calipers: head width (HeadW); head length (HeadL); interlimb length (ILimbL);

213   hindlimb length (HindLL). Left and right measurements were averaged to obtain one

214   measurement for that trait. We also recorded snout–vent length (SVL), total length,

215   and mass (see Table S1 for further details). All measurements were taken by one

216   person (SLM) to minimize observer bias. All morphological variables were log-

217   transformed prior to regression analyses.

218

219   <u>Climatic variables, and connectivity</u>

220   As our study aimed to assess adaptation to local climate, various temperature and

221   precipitation variables were extracted for each site (see Table S2 for details). We

222   considered both means and extremes. It is important to consider climatic extremes,

223   because temperature extremes may be increasing faster than mean temperatures (30),

224   and many species are likely to be particularly vulnerable to these extremes (31). Many

225   environmental variables are highly correlated (28), so only the less-derived variables

226   were used in analyses, specifically: annual mean precipitation (AMP); seasonality of

227   precipitation (Pcov); precipitation of the driest quarter (Pdry); annual mean

228   temperature (AMT); coefficient of variation of temperature (Tcov); average minimum

229   daily temperature (Tmin); average maximum daily temperature (Tmax); average

230   variance of daily maximum temperature (TmaxVar); and average variance of daily

231   Tmin (TminVar).

232

233

234   Our connectivity index was designed to capture the flux of individuals through a

235   location and is detailed in the Supplementary Material. Briefly, it is a measure of

236   habitat suitability for our focal skink species, averaged over space using a species-

237   specific estimate of dispersal potential. As our species is an obligate rainforest-

238   dweller, grid cells in the landscape that are rainforest and that are surrounded by

239   rainforest have high connectivity indices, while grid cells of rainforest surrounded by

240   non-rainforest matrix have low indices. See Table S2 for further details on all

241   variables, and Figure S1 for correlations between all variables.

242

243   <u>Analysis</u>

244   Our analysis aimed to assess: 1) the relationship, across sites, between each trait and

245   each environmental variable; and 2) how connectivity affected each of these

246   relationships (i.e., the interaction between connectivity and environment). To allow

247   comparison of coefficients across variables, and to make interaction effect-sizes

248   meaningful, all trait and environmental variables were standardized so they had a

249   mean of 0 and a standard deviation of 1. Linear models were fitted for each pair of

250   environment–trait variables, with all models including the effect of lizard body size

251   and sex, as well as the interaction between environment and connectivity:

252     $\text{trait}_i = A + B_{svl} \times \text{SVL}_i + B_{sex} \times \text{Sex}_i + B_{env} \times \text{Env} + B_{conn} \times \text{Conn} + B_{int} \times \text{Env} \times \text{Conn} + error_i$

253   Where:

254   trait_i = standardized trait value of interest for lizard i

255   A = intercept

256   B_{svl} = coefficient of SVL

257   SVL = lizard snout–vent length, to control for effect of body size

258   B_{sex} = coefficient of Sex

259   Sex = lizard sex (this species is sexually dimorphic in some morphological

260   traits)

261   B_{env} = coefficient of environmental variable

262   Env = environmental variable (e.g., annual mean temperature) at the lizard's

263   site

264   B_{conn} = coefficient of connectivity

265   Conn = connectivity index at the lizard's site

266   B_{int} = coefficient of interaction between Env and Conn

267        $error_i$ = deviation between expected value and trait value of lizard i

268

269   A score for ranking the strength of local adaptation was then calculated as:

270                                    $$L = -B_{env}\, B_{int}$$

271   If the signs of the two coefficients (B_{env} and B_{int}) are opposite (which indicates an

272   environmental effect being eroded by high connectivity, i.e., evidence for local

273   adaptation), L will be positive. If the signs are the same (which indicates an

274   environmental effect being enhanced by high connectivity, a situation not consistent

275   with local adaptation), L will be negative. Thus, higher numbers on this scale equate

276   to stronger evidence for local adaptation in that environment–trait pair. This score

277   can, in theory, range from $-\infty$ to $+\infty$. Once many environment–trait combinations

278   have been assessed, the coefficients for all pairs can be plotted (see Fig. 1). As

279   described in the Introduction, in a system dominated by local adaptation, we expect to

280   see a negative relationship between B_{env} and B_{int} (Fig. 1B). All analyses were

281   conducted in R v3.2 (32).

282

283   **Results**

284   There was substantial variation in the effect of environment (B_{env}) and its interaction

285   with connectivity (B_{int}) across climate and trait variables, with B_{env} ranging from -1.64

286   to 1.37, and B_{int} ranging from -0.89 to 0.74 (Fig. 3). Despite this variation, a clear

287   pattern is evident, with most points in Figure 3 appearing in the top-left or bottom-

288   right quadrants: the quadrants in which the two coefficients have opposing signs, and

289   where we would expect points to fall if environment–trait relationships are due to

290   local adaptation. Across these environment–trait combinations there is a distinct

291   negative linear trend (slope= -0.36, p < 0.001). It is especially noteworthy that the

292   environment–trait pairs with the largest coefficients (i.e., those points furthest away

293   from the origin) are in the two quadrants indicative of local adaptation.

294

295   Overall, physiological traits showed substantially stronger environmental effects (i.e.,

296   larger values of B_{env}) than did morphological traits, with the largest environmental

297   effects being exhibited by CTmin (AMP: -1.64; Tmax: 1.31; Pdry: 1.28) and CTmax

298   (Pdry: 1.37). Physiological traits also showed stronger interactions between

299   environmental effects and connectivity, again with CTmin and CTmax showing the

300   largest interactions. These trends are apparent when we examine our index of local

301   adaptation, L. Figure 4 shows a heatmap of all environment–trait pairs, ranked via

302   reciprocal averaging according to the strength of their local adaptation index. The

303   environment–trait pairs that show the strongest signature of local adaptation appear at

304   the top-left in red. There is a rough divide, with most of the physiological traits on the

305   left and most of the morphological traits on the right. The exceptions are the

306   physiological traits Topt and Rmax, which appear at the far right of the figure.

307

308   The two environmental variables that produced the strongest effects (topmost rows in

309   Fig 4) were both precipitation related: annual mean precipitation (AMP) and

310   precipitation of the driest quarter (Pdry). In our system, AMP and Pdry are both

311   highly correlated with connectivity (see Fig. S1). This is expected, because our

312   connectivity index is largely a measure of where rainforest is, and the distribution of

313   rainforest in our study region is driven to a large degree by rainfall.

314

315

316   **Discussion**

317   Understanding environment–trait correlations will help us plan management

318   strategies, such as targeted gene flow (TGF), that can mitigate the impact of climate

319   change on vulnerable species. Numerous studies have looked for (and found)

320   environment–trait correlations (e.g., 18, 19, 33, 34-36), but the interpretation of these

321   associations is plagued with uncertainty: are they associations due to local adaptation,

322   or plasticity? By acknowledging that gene flow undermines adaptation, we can

323   incorporate connectivity (a proxy for the flux of genes) into our analysis, and in doing

324   so, separate correlations due to local adaptation from those due to plasticity.

325

326   <u>Local adaptation</u>

327   In the environment–trait combinations we assessed, physiological traits typically

328   showed a substantially stronger effect of environment than did morphological traits,

329   with the largest environmental effects shown in CTmax and CTmin (Figs. 3 & 4).

330   Physiological traits also generally showed stronger environment–connectivity

331   interactions, again with CTmin and CTmax showing the largest interactions. Overall,

332   physiological traits generally showed stronger evidence of local adaptation than did

333   morphological traits. This result is intuitive: we would expect an ectotherm's

334   physiological traits to be under strong selection from climate (37-39), but the fitness

335   link between morphology and climate is much less clear. Had we also included some

336   environmental variables that had a clearer bearing on morphology, we might have

337   detected stronger environment–trait relationships for morphology. For example,

338   skinks that occur in rockier habitats show various morphological adaptations to that

339   environment (40). Including a measure of rockiness in our set of environmental

340   variables, for example, might have allowed us to detect environmental correlations

341   with limb length.

342

343   Of the environmental variables used, our analysis suggests that precipitation is a very

344   strong driver of local adaptation, even in thermal traits that might not seem obviously

345   related to precipitation (e.g., CTmin, CTmax). Although this may seem a surprising

346   result, precipitation has been shown to directly affect growth rate, body temperature,

347   activity patterns, and thermoregulatory opportunities in lizards (38, 41-45). Wetter

348   areas also have higher thermal inertia (and so lower cyclical thermal fluctuations

349   (46)), and changed environmental variance in temperature potentially has a strong

350   influence on thermal limits (47). Additionally, Bonebrake and Mastrandrea (48) found

351   that changes in precipitation can significantly affect modeled fitness and performance

352   curves. Finally, comparative analyses also suggest that precipitation can influence

353   thermal traits in many species (38). Thus, although the mechanisms linking

354   precipitation to thermal limits are diffuse and poorly resolved, they do exist, and our

355   analyses suggest that precipitation is a strong driver of local adaptation at thermal

356   physiological traits.

357

358   Our analysis also suggests that temperature is an important driver of local adaptation

359   in this system, but that extremes of temperature (encapsulated in minimum and

360   maximum temperatures) are at least as strongly associated with local adaptation as is

361   mean temperature. Again, this result is intuitive (natural selection from climate is

362   likely stronger during extreme events than during normal daily temperatures) and

363   agrees with results of empirical studies (38). Finally, the environmental variables with

364   the weakest signals of local adaptation are Tcov (temperature seasonality), TminVar,

365   and TmaxVar (variance of minimum and maximum daily temperatures, respectively).

366   These variables represent predictable environmental variation occurring within an

367   individual's lifespan and so are variables to which we might expect individuals to

368   develop plastic responses, rather than adaptive responses (49-51).

369

370   <u>System-wide signal of local adaptation</u>

371   The clear negative linear trend displayed in Fig. 3 is precisely what we would expect

372   in a set of environment–trait combinations dominated by local adaptation. Migrant

373   load (the negative effect of the immigration of less-locally adapted individuals) scales

374   positively with immigration as well as with the strength of selection [see equation 5 in

375   Polechová, Barton and Marion (52)]. The reason for this is that, when the strength of

376   selection is moderately high, the environment will have a large effect on relevant

377   traits, and therefore any immigrants coming from differing environments will be

378   particularly maladapted and will therefore have a large and negative impact on the

379   local phenotype. Thus, we expect environment–trait combinations with strong local

380   adaptation to show strong effects of connectivity on the environment–trait correlation

381   (52).

382

383   We used long-term climatic averages and found strong evidence that local adaptation

384   dominates over plasticity in our system. If we had included different environmental

385   variables, such as the conditions the lizards had recently encountered, signals of

386   plasticity may have been more apparent. Clearly environmental variables that are

387   similar across generations should lead to local adaptation, while environmental

388   variables that fluctuate within generations should have a strong influence on

389   phenotypic plasticity

390

391   <u>Phenotypic plasticity</u>

392   The importance of accounting for phenotypic plasticity is exemplified in our dataset

393   by the relatively strong effect of precipitation of the driest quarter (Pdry) on the

394   temperature at which maximum sprint speed is achieved (Topt) and on maximum

395   sprint speed (Rmax) itself. On their own, these strong correlations might traditionally

396   be considered evidence for local adaptation. Our analysis, however, suggests that the

397   environmental effect is largely unaffected by connectivity, implying that variation in

398   these traits is due to plasticity. Other work (28) has shown little temporal variation in

399   Topt (within generations) despite clear geographic variation and this, together with

400   our results, suggests that this trait undergoes developmental plasticity, but is fixed in

401   adult lizards. In principle, this non-effect of connectivity could also arise due to

402   selection that is so strong that it maintains local adaptation despite high levels of gene

403   flow [i.e., immigrants are selected against so strongly that they do not contribute to

404   the effective population (11)]. The environment–trait relationships for Topt and Rmax

405   are, however, weaker than those for some other traits (e.g., CTmax and CTmin) that

406   show clear effects of connectivity, so extremely strong selection seems an unlikely

407   explanation for the pattern we see here.

408

409   <u>Assumptions and limitations</u>

410   Our analysis requires an index of population connectivity across the landscape,

411   something that can be calculated relatively easily for many species by using broad

412   scale habitat mapping datasets [e.g., vegetation mapping from DERM (53)]. Where

413   possible, these measures of connectivity should be calculated at a scale relevant to the

414   scale of dispersal of the species in question [as was ours, using dispersal rate data for

415   *Lampropholis coggeri* from Singhal and Moritz (54)]. For highly mobile species, such

416   as plants or insects that have wind-assisted dispersal and for which calculating

417   connectivity between populations may be difficult, it is possible our approach will not

418   work. But for many species of animals with relatively low vagility (i.e., the species

419   for which TGF is most needed), our approach should be generally applicable.

420

421 Our approach implicitly assumes that traits have a linear response to the environment

422 (at least at the environmental scale across which we are looking). In most instances,

423 this will be a reasonable assumption: it seems unlikely, for example, that a trait such

424 as desiccation resistance would be high in dry environments, low in moderately wet

425 environments, and then high again in very wet environments. The reason our

426 assumption of linear environment–trait relationships bears mentioning is that a

427 limitation of our technique arises when the connectivity index is strongly correlated

428 with one or more of the other environmental variables being used. In our system, for

429 example, AMP and Pdry are correlated with connectivity (Fig. S1). Where the

430 environment–connectivity correlation is very strong, the interaction term in our model

431 (Conn×Env) could be interpreted as a quadratic term for environment (i.e., Env^2). In

432 these cases, it is possible that a strong connectivity interaction is, in fact, pointing to a

433 non-linear environment–trait relationship. Thus, for environmental variables that

434 correlate with connectivity (and there will always be some), careful consideration

435 needs to be given to the possibility of a quadratic fitness function between

436 environment and trait. In our case, it remains possible, for example, that the strong

437 influence of precipitation on local adaptation in our system is spurious, and instead

438 reflects non-linear relationships between optimal trait values and precipitation. We

439 can, however, think of no obvious reason why thermal limits should respond

440 quadratically to precipitation, nor why desiccation rates and other physiological traits

441 should also do so. Thus we are inclined to accept the importance of this

442 environmental variable in driving local adaptation in our system.

443

444   Finally, our approach, by examining one environment–trait combination at a time,

445   may potentially miss relationships that only appear in multivariate analyses. For

446   example, if two environmental variables are negatively correlated but both have a

447   positive effect on a trait, it is possible that these countergradients can obscure the

448   univariate relationship. Similar problems are encountered when examining response

449   to selection over time (55), and with our approach may lead us to underestimate the

450   number of important environmental drivers of local adaptation. To minimize this

451   effect, care should be taken in future work to sample environmental spaces in such a

452   way as to minimize correlations between environmental variables. Such an aim can be

453   achieved by, for example, strategically exploiting latitudinal and altitudinal gradients.

454

455   Conclusion

456   There is increasing urgency to identify populations that will act as suitable sources for

457   targeted gene flow efforts in the face of climate change. To identify these populations,

458   we need to know which traits influence sensitivity to climate and are locally adapted.

459   Traditional approaches to unearthing local adaptation (reciprocal transplants and

460   common garden experiments) are time consuming, and often cannot attribute

461   adaptation to any particular environmental driver. Local adaptation is, however,

462   undermined by gene flow, and we should be able to use this fact to sort patterns of

463   local adaptation from patterns with other causes. Here we have demonstrated this

464   approach: using connectivity as a proxy for gene flow, and looking for its effect on

465   environment–trait correlations. Our analysis, using a species of lizard from Australia's

466   Wet Tropics rainforest, suggests the approach has merit: the results we achieve are

467   coherent and suggest local adaptation is the overwhelming signal in the set of

468   environment-trait correlations tested. As well as confirming a strong role for local

469   adaptation, we have effectively ranked environmental drivers of local adaptation,

470   finding evidence that precipitation and temperature are important environmental

471   variables with regard to local adaptation. Our analysis also suggests that some traits

472   exhibit strong plastic responses to the environment, particularly in response to

473   precipitation of the driest quarter and the seasonality of temperature and precipitation.

474   These specific results will likely apply to other species that are phylogenetically or

475   ecologically similar to our focal species, but the method has the potential to apply

476   much more broadly. Given the potential of this method to provide evidence of local

477   adaptation, and rapid ranking of the climatic drivers of local adaptation, assessment of

478   the method in a broader array of systems is warranted.

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Figure Captions

Figure 1. Graphs showing the concepts illustrated by plotting a set of environment–trait coefficients (x-axis) and the corresponding environment–connectivity interaction coefficients (y-axis). A) Phenotypic plasticity is suggested when environment–trait relationships are strong, but are not influenced by connectivity. B) Local adaptation is suggested when increasing connectivity diminishes the correlation between the environment and the trait. C) The effect of the environment is enhanced by connectivity. This latter pattern might arise in organisms that are highly mobile and can actively move to their ideal environment, thus avoiding the selective pressures that would lead to local adaptation.

Figure 2. Map of the southern Australian Wet Tropics bioregion, showing the distribution of rainforest and the sampling locations.

Figure 3. Scatterplot showing the results of 99 linear models run to assess the relationship between each pair of environment–trait variables and the environment–connectivity interaction. Environment–trait coefficients are on the x-axis, and environment–connectivity interaction coefficients are on the y-axis. Local adaptation is suggested in environment–trait pairs that show a strong environmental effect that is eroded by increasing connectivity.

Figure 4. Heatmap showing the relative rankings of climate variables (rows) and morphological and physiological traits (columns). The matrix has been sorted (by reciprocal averaging) and coloured according to the strength of local adaptation, with higher values coloured red and being sorted to the top/left. See Tables S1 and S2 for explanations of the trait and environmental variables used.

L = local adaptation index: $-B_{env} B_{int}$

B_{env} = coefficient of the environmental variable.

B_{int} = coefficient of the interaction between the environment and connectivity terms.
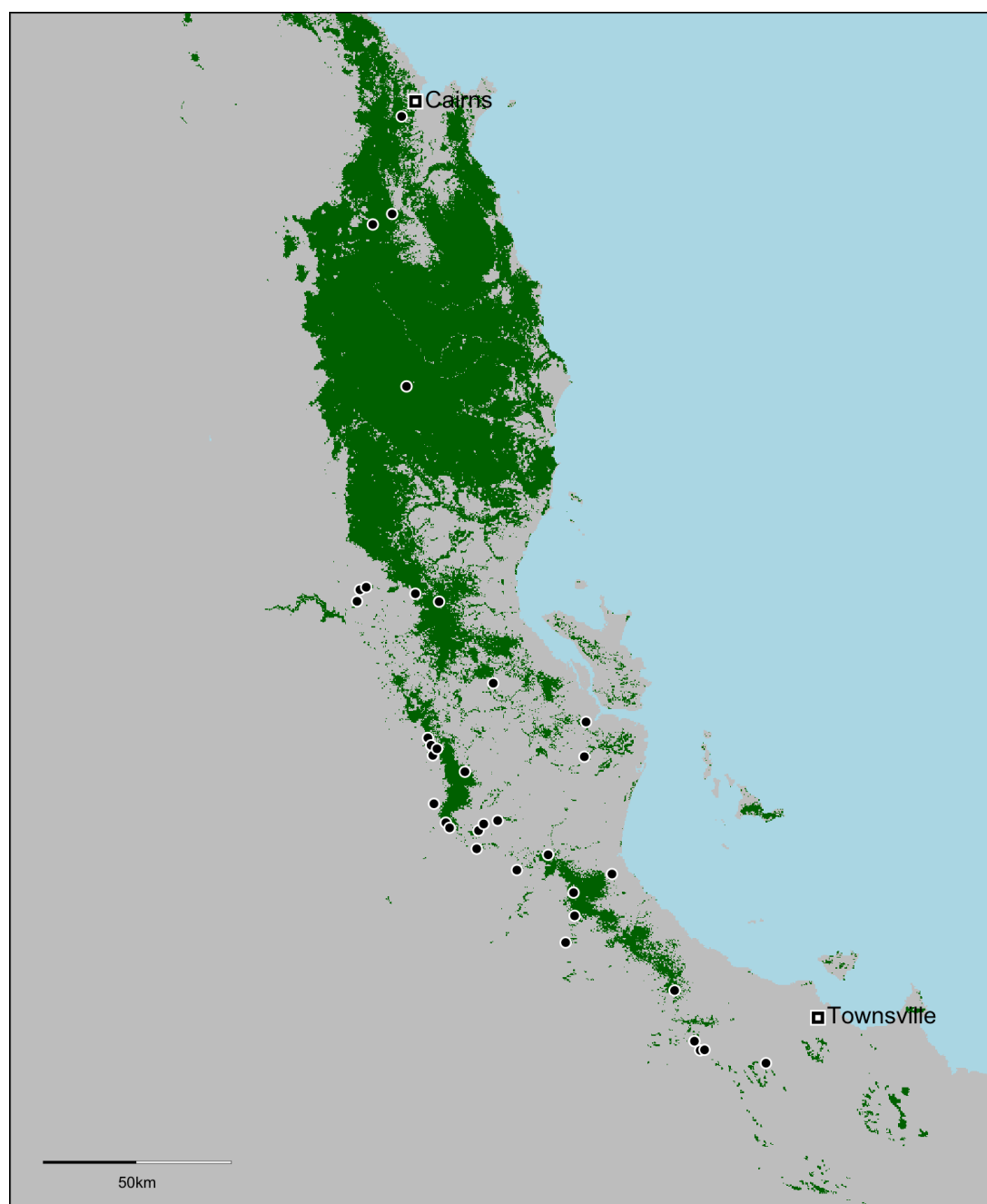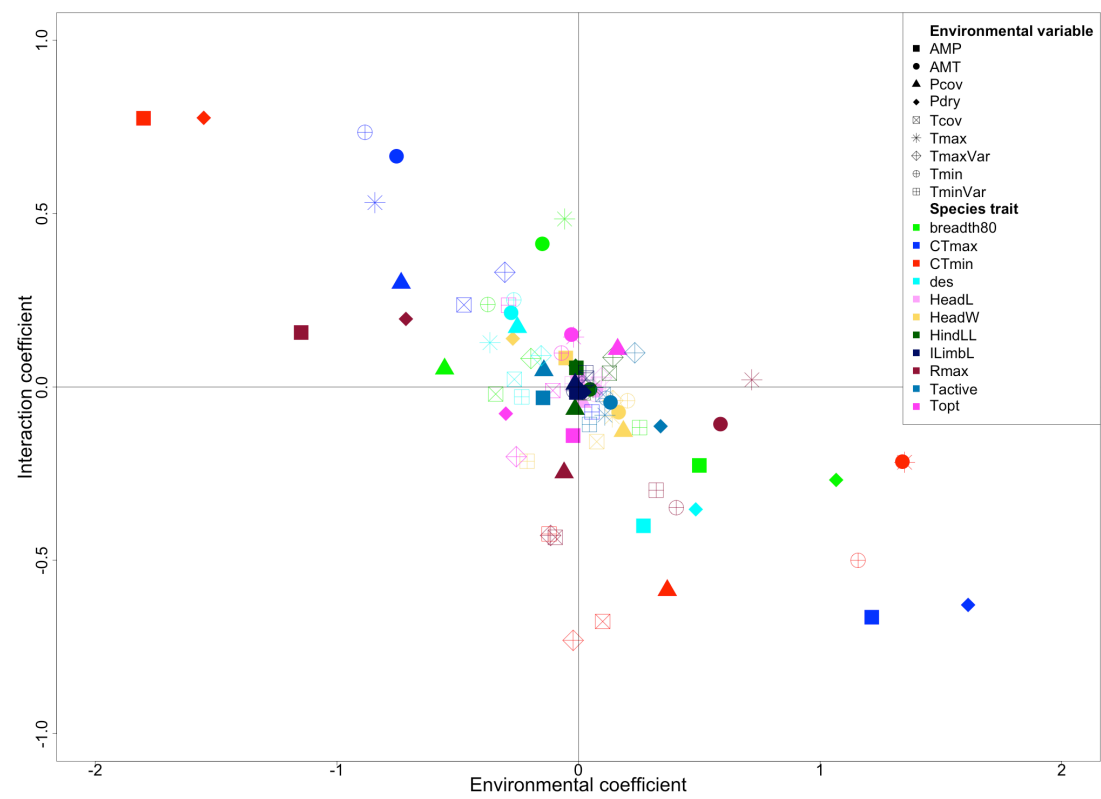
674 **Figures**
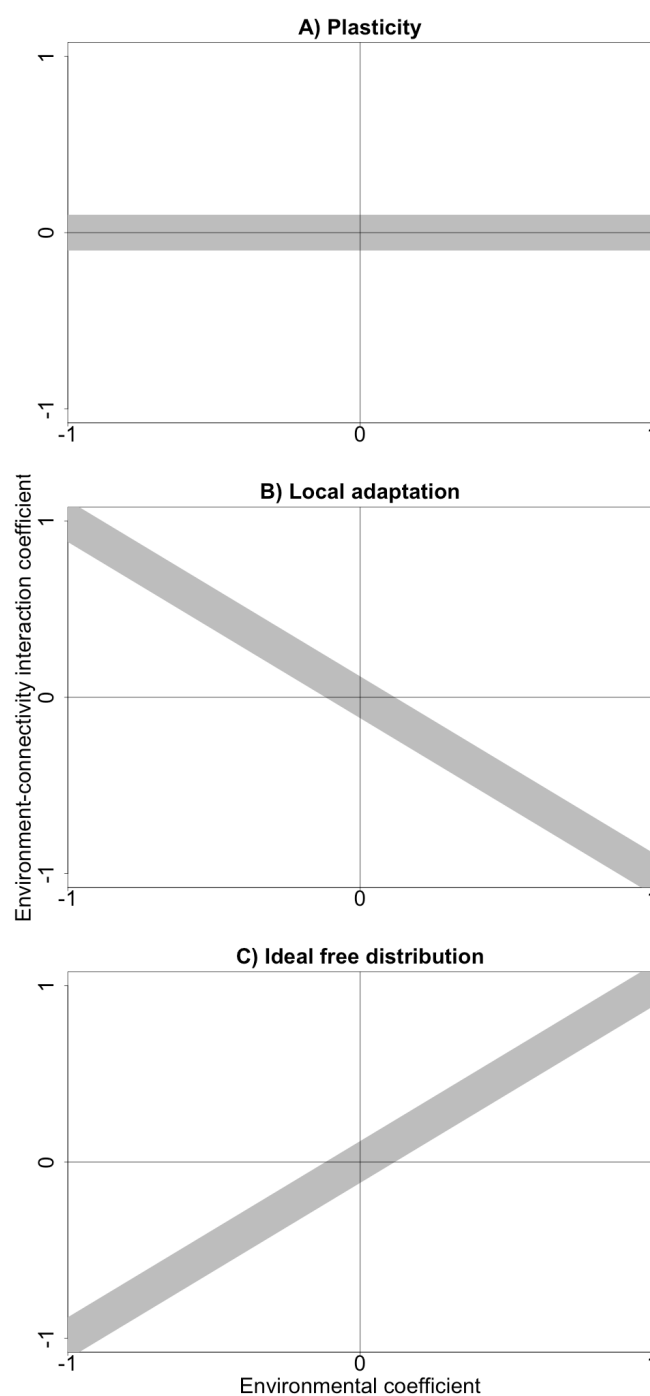
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676 **Figure 1**

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679 **Figure 2**

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684 **Figure 3**

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	CTmin	CTmax	breadth80	Rmax	des	HeadW	Tactive	HindLL	HeadL	ILimbL	Topt
AMP	L = 0.7751	L = -0.6644	L = -0.2265	L = 0.1570	L = -0.4007	L = 0.0835	L = -0.0315	L = 0.0551	L = 0.0219	L = -0.0159	L = -0.1404
Pdry	L = 0.7763	L = -0.6289	L = -0.2683	L = 0.1961	L = -0.3532	L = 0.1395	L = -0.1134	L = 0.0613	L = 0.0279	L = -0.0150	L = -0.0771
Tmin	L = -0.5004	L = 0.7345	L = 0.2380	L = -0.3484	L = 0.2510	L = -0.0399	L = -0.0329	L = -0.0157	L = -0.0219	L = -0.0116	L = 0.0977
AMT	L = -0.2156	L = 0.6655	L = 0.4128	L = -0.1072	L = 0.2139	L = -0.0730	L = -0.0447	L = -0.0071	L = -0.0099	L = -0.0043	L = 0.1511
Tmax	L = -0.2173	L = 0.5316	L = 0.4846	L = 0.0206	L = 0.1275	L = -0.0864	L = -0.0813	L = -0.0020	L = -0.0041	L = 0.0002	L = 0.1438
Pcov	L = -0.5868	L = 0.2996	L = 0.0522	L = -0.2473	L = 0.1719	L = -0.1279	L = 0.0474	L = -0.0642	L = -0.0440	L = 0.0082	L = 0.1089
Tcov	L = -0.6766	L = 0.2366	L = -0.0206	L = -0.4338	L = 0.0219	L = -0.1579	L = -0.0219	L = 0.0397	L = 0.0083	L = 0.0253	L = -0.0104
TmaxVar	L = -0.7312	L = 0.3308	L = 0.0820	L = -0.4284	L = 0.0898	L = -0.0394	L = 0.0984	L = 0.0853	L = 0.0267	L = 0.0034	L = -0.2013
TminVar	L = -0.4241	L = -0.0721	L = -0.1174	L = -0.2979	L = -0.0289	L = -0.2144	L = -0.1083	L = -0.0172	L = 0.0095	L = 0.0409	L = 0.2357

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688 **Figure 4**

689